Identification and Characterization of a Novel Rat Triosephosphate Isomerase Gene in Remnant Ileum After Massive Small Bowel Resection

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This paper describes the identification and characterization of a novel cDNA encoding a putative protein of 254 amino acids that is highly homologous to triosephosphate isomerase. The cDNA was isolated by subtractive hybridization and was differentially expressed in the remnant rat ileum after massive small bowel resection. The novel triosephosphate isomerase was named rsTPI (resection-induced TPI) and the putative protein encoded RSTPI. The nucleotide and amino acid sequences of rsTPI and RSTPI were about 60% and 62% homologous to Giardia lamblia TPI and TPI, respectively. Active catalytic sites (Lys 13, His 95, and Glu 167) and the peptide motifs, AYEPVWSIGT and GGASLKPEF found in other triosephosphate isomerases were conserved in RSTPI. rsTPI expression was detected in normal ileum and pancreas by reverse transcription-polymerase chain reaction. Expression of rsTPI in remnant rat ileum was detectable by northern blot analysis one week after massive small bowel resection. Expression increased significantly by 2.8-fold between one and two weeks after surgery. High levels were maintained for at least one month after surgery. The up-regulation of triosephosphate isomerase expression in the remnant small intestine after massive resection indicates that it may play an important role in the adaptive process.

KEY WORDS: differential expression; subtractive hybridization; intestinal adaptation; triosephosphate isomerase; small bowel resection.

After massive small bowel resection, the remnant intestine undergoes compensatory morphologic and functional changes (1-4). A rat model for studying intestinal adaptation has been well established (5). Massive small bowel resection (MSBR) provides a potent stimulus for growth of the remnant intestine. To compensate for the loss of absorptive surface area, the remnant intestine adapts mainly by the proliferation of intestinal crypt cells. In the process of adaptation, the remnant gut epithelium becomes markedly hyperplastic and displays increased villus height and crypt depth as well as dilation and lengthening of the intestinal remnant (6). These changes offer an excellent model to study mechanisms involved in growth and differentiation of the intestine (7). Moreover, the absorptive capability per unit length also increases aided by decreased gut motility (8). Although the morphological changes in the adaptive process have been well documented, the molecular mechanisms underlying intestinal adaptation are not well understood. Luminal nutrition, pancreatiobiliary secretion, and humoral and luminal growth factors and hor-
mones have all been implicated (9, 10). However, none of these appear to be essential as mediators of the adaptive response, indicating the involvement of a complex set of molecular pathways (9). To better understand the molecular aspects of the adaptive response, we utilized a subtractive hybridization strategy to isolate genes differentially expressed in the small intestine after massive resection. This approach may provide insights into the identity of potential mediators and modulators of the adaptive response. This paper describes the isolation and characterization of one of the differentially expressed genes.

MATERIALS AND METHODS

Animals and Surgery. Tissues used for subtractive hybridization experiments were obtained from two male Sprague-Dawley rats (250 g). Following an overnight fast, they were anesthetized by an intraperitoneal injection of pentobarbital sodium (Nembutal, 60 mg/kg body weight). Under sterile conditions, the abdomen was opened by a midline incision and the small intestine exteriorized. One of the two rats underwent a 70% intestinal resection of the small intestine, leaving 1 cm of the jejunum distal to the ligament of Treitz and 5 cm of the ileum proximal to the ileocecal junction. Intestinal continuity was restored by a single layer anastomosis with interrupted 5/0 silk sutures. The small intestine of the other rat was transected at 10 cm proximal to the ileocecal junction with reanastomosis using 5/0 silk sutures. All incisions were closed with interrupted 4/0 silk sutures. At the end of the surgery, both animals were given 18 mg Cephalin (Ceporex, Pittman-Moore) subcutaneously. On the first postoperative day, the rats were given an isotonic solution of 0.9% saline/5% glucose. From the second postoperative day onwards, the animals were given free access to pelleted rat chow and tap water. Both rats were killed by decapitation seven days after the surgery, and 5 cm of the ileum beginning 1 cm distal to the site of anastomosis was removed. The luminal contents were flushed with 0.9% cold saline, and the ileal tissues were snap-frozen in liquid nitrogen and stored at −80°C.

To determine gene regulation after massive small bowel resection (MSBR), 36 male adult Sprague-Dawley rats (180–220 g) were used. Rats were randomly divided into two groups. One group (N = 18) underwent a 70% resection of the small intestine, while the other group (N = 18) underwent transection operations as described above. The rats were killed at 2 days (N = 10), and 1 (N = 8), 2 (N = 8) and 4 (N = 10) weeks after surgery by decapitation. At each time point, half the rats had undergone resection, while the other half had undergone transection surgeries. Beginning 1 cm distal to the anastomosis site, 5 cm of the ileum was removed at sacrifice. The tissues were immediately frozen on Dry Ice and subsequently stored at −80°C. To study gene expression in various tissues, the pancreas, liver, spleen, lung, kidney, adrenal gland, skeletal muscle, heart, testis, hypothalamus, cerebellum, stomach, duodenum, jejunum, ileum, cecum and colon were removed from one rat after decapitation.

Differential Subtraction. Subtractive hybridization was carried out to identify genes that were up-regulated seven days after MSBR. Total RNA was isolated as previously described (11) and enriched for poly(A) RNA using pre-packed oligo(dT)-cellulose spun columns (Pharmacia, Uppsala, Sweden). Then 2 μg of mRNA from each experimental sample was subjected to subtractive hybridization using the PCR-Select cDNA Subtraction Kit (Clontech) following the manufacturer’s protocol. Briefly, mRNA from the transected and resected ileum was reverse transcribed at 42°C for an hour using MMLV reverse transcriptase and the cDNA synthesis primer provided in the kit. The cDNAs from both tissues were digested with RsaI to yield shorter, blunt-ended fragments. The population of cDNA fragments from the ileum of the resected animal (tester) was sub divided into two portions. Each portion was ligated to a different adaptor. Two rounds of subtractive hybridization were then performed. The entire population of molecules was then subjected to suppression polymerase chain reaction (PCR) using the primers and PCR parameters recommended. A secondary PCR amplification was then performed using nested primers to enrich for differentially expressed sequences. The PCR-amplified fragments were ligated into a pMOSBlue T-vector and transformed into MOSBlue competent cells (Amersham, Buckinghamshire, England). The fragments were sequenced using ThermoSequenase Radiolabeled Terminator Cycle Sequencing Kit (Amersham) and CastAway Precast Sequencing System (Stratagene, La Jolla, California).

Preparation of Probe and Northern Blot Analysis. Sequence analysis revealed that one of the clones contained sequences homologous to Giardia lamblia TPI. The regulation of gene expression was studied by northern blot analysis. The novel TPI cDNA was amplified by PCR and gel-purified (Qiagene, Qiagen, Chatsworth, California). The purified fragment was labeled with 32P using the Rediprime DNA Labelling System (Amersham) and used as hybridization probe. Total RNA from the resected and transected rat ileum as well as other rat tissues was prepared using the guanidium thiocyanate method (11). RNA (15 μg) was separated by formaldehyde–agarose gel electrophoresis, transferred to nylon membrane (Qiabrance, Qiagen), cross-linked (Stratalinker, Stratagene), and hybridized to the novel cDNA probe. The 18S rRNA oligonucleotide (5'-GACAAGCATATGCTACTGGC-3') (12) was synthesized using a Beckman 1000M oligonucleotide synthesis machine. The oligonucleotide was end-labeled with 32P using T4 polynucleotide kinase (Promega, Madison, Wisconsin). After labeling, the unincorporated nucleotide was purified through a Qiaquick nucleotide removal kit (Qiagen). Minor variations in loading of total RNA were corrected by hybridization to the 18S oligonucleotide probe after the blot was stripped off the cDNA probe. For hybridization to cDNA probe, the blots were prehybridized in 6 × SSC (0.9 M NaCl, 0.09 M Na₃ citrate · 2H₂O), 50% formamide, 0.5% sodium dodecyl sulfate (SDS), and 100 μg/ml denatured, fragmented salmon sperm DNA for 8 hr at 50°C. Denatured cDNA probe was then added to the prehybridization mix to a concentration of 0.5 × 10⁶ cpm/ml and hybridized overnight at the same temperature. The blots were then washed twice in 2 × SSC/0.1% SDS at room temperature for 15 min each and twice in 0.2 × SSC/